# MASTER THESIS

research on the epidemiological analysis of breed-related inherited diseases and harmful breed specifications of dog breeds in the Netherlands


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# Abstract

Breed-related disease and harmful breed specifications are a big problem in the global dog breeding sector, also in the Netherlands. It is clear that rigorous change in breeding is necessary in order to improve the long term well-being of purebred dogs. Only after identification and quantification of a breed's health problems, further measures as modern DNA diagnostics and epidemiological techniques can be deployed to collectively and systematically develop new effective breeding policies. A primary need to effectively improve breed health, is that breeders have insight in the incidence of disease and of harmful breed characteristics in their breed population.

The 'Expertise Centre Genetics of Companion Animals' (Faculty of Veterinary Medicine, Department of Clinical Sciences of Companion Animals of Utrecht University), that centers all scientific knowledge and research on veterinary genetics, population genetics and molecular genetics, aims to create this detailed insight in the status of breed-related health problems in the Netherlands. In the here reported research project, the progressive process is described of creating, thoroughly organizing and analyzing a disease registration database of dogs in the Netherlands.

Qualitative analysis using existing literature, current veterinary expert opinions and the patient database of the University Clinic for Companion Animal Health of Utrecht University, gives insight in the health status of breeds. However, these data should be combined with quantitative data to draw reliable conclusions regarding the health of an entire breed population. Therefore, disease registrations from primary care veterinary practices were collected. These were automatically sent to and monitored in a standardized, cumulative and world-leading database showing diseases incidence in all breed populations. The aims of this Master thesis were (1) to set up a new standard for reporting qualitative analyses of diseases and harmful breed characteristics in Dutch purebred dog populations, and (2) to analyze recorded first line practice diagnostic data in the central database and develop an structured method to analyze these large scale data which could be used for future quick scan analysis of many populations.

The database was thoroughly organized as such, that it generates a fast overview of the most problematic health issues for every breed. However, to indicate an overrepresentation of a breed within a disease, these data should be further analyzed. This can be done by comparing the incidence of a specific diagnosis, sub-diagnosis or involved organs system in breeds to this incidence in mixed-bred dogs. Another possibility is to use a method called data mining. This scientific analysis searches for statistical relations and/or patterns within a voluminous dataset. This method is time-consuming and complex, but prevents data exclusion and should therefore generate reliable results. As soon as data are accessible, these methods will indicate all overrepresentations in any breed, generating an evidence-based overview of the health status of breeds in the Netherlands.

This project has resulted in a new format for standardized reporting of qualitative data of companion animal breed populations. Furthermore, a matrix for quantitative analysis of large scale first line diagnostic data was developed which may be employed for future quick scan data analysis. On the long term, this may contribute to new, evidence-based, breed-specific and durable breeding policies that improve and maintain the health of pedigree animals in the Netherlands.

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# Introduction

## 1.1. Motivation

Breed-related health problems in pet breeds has grown awareness in both public debate and media [1–5] as well as in breeding and governmental authorities [6,7] within the Netherlands. Due to breeding methods used in the last 150-200 years (selection based on externally observable qualities or behavior of the animal), unintentionally, most breed populations suffer at least one (inherited) disease or disorder that appeared in direct relation to this selection policy[8]. Also, some favored breed characteristics have become exorbitant over generations of selection, becoming harmful for the animal itself: so-called harmful breed specifications. It is clear that rigorous change in pet breeding is necessary in order to improve their long term well-being. In the last couple of years, increasing social pressure and developed molecular-genetic knowledge has led authorities to force all parties in the Dutch breeding sector to take their role in working towards more durable and healthier pet breeds.

## 1.2. Background

### 1.2.1. Inherited diseases

Hundreds of inherited diseases and disorders have already been described for dog and cat breeds throughout the world. In 2010, research on all existing literature reported 312 breed-related diseases in dogs only[9]. Recently, the number of known disease mutations in the genome of dogs has risen up to more than 600[10].

Of course breeders do not intend to produce sick offspring. However, constant selection based on specific breed characteristics, automatically means selection for DNA-variations or mutations that are coupled and localized on the same chromosome as the favored genetic aspect. So, unintentionally, selection also enables mutations that might cause disease to be transmitted onto the next generation. These mutations are present in nonselective open populations as well, but reveal themselves at very low frequencies. A breed, however, is a closed population of which only a small percentage is selected to produce offspring. This selection is most likely to consist of the animals that best resemble the characteristics as set in the breed standard. This means that the so-called effective population size is much smaller than the total number of pedigree animals that belong to this breed population[11,12]. Also, many breeds originally derive from a small group of (closely) related parent animals, leaving these breed populations with only limited genetic variation (heterogeneity). This genetic 'bottle neck' creates a higher risk for specific gene combinations to occur that express clinical disease. This is the reason dog breed populations deal with highly increased frequencies of inherited diseases[8,9,13]. These diseases are expressed in 5-50% of the population, which accounts for a frequency up to 1000 times higher than is expected in an open population (such as humanity).

Also, inherited disease can arise by random mutations in the genome of a stud. Frequent use of this same stud enables this gene mutation to spread widely into the population rather quickly. This may lead to high incidence of disease within the breed, known as the 'popular sire' effect. Moreover, in case of a recessive or a complex polygenic inheritance pattern, genetic carriers of the disease are not phenotypically recognized[14,15]. These individual animals are responsible for the conservation of the disease within the population. Lastly, inherited disease with reduced penetrance or a multifactorial background lead to such a variation of symptoms that a

genetic cause may seem unlikely. In addition to this, inherited disease is often expressed at higher age, at which the animal may already been used for breeding and thus passed the genetic predisposition on to offspring.

#### 1.2.2. harmful breed characteristics

A harmful breed characteristic is an external trait that is part of the breed standard and therefore actively pursued by breeding, which eventually lead to an exaggerated form of is this trait causing direct harm to the animals' welfare. The difficulty in this matter, lies in the fact that it involves a 'slippery slope'; the trait that is present throughout the population is not necessarily harmful, but may get emphasized by selection in such an extent that dysfunction is caused for the animal[16]. Recent research showed the extent of this problem, reporting at least 84 diseases and disorders being a direct result of external breed characteristics[15].

Ethics play an important role in handling harmful breed characteristics, and widely present in the public debate on animal breeding. It Is important to use objective criteria to measure and assess the possible harm of a breed characteristic. The Expertise Centre Genetics of Companion Animals within the Utrecht University has defined the following criterion: a breed characteristic is harmful, when the owner has to appeal upon a veterinarian to treat or remove its harmful effects. This enables assessing and measuring if bred animals are consciously damaged by their existence, therefore exceeding ethical borders.

This background shows that it is impossible to effectively reduce genetic diseases in breed populations using only clinical diagnostics. In case rigorous measures are applied to fight breed-related health disease, this often leads to new problems. For example, when not only affected animals but also fairly related animals are excluded from breeding programs, the subsequent loss of genetic heterogeneity will eventually lead to new genetic problems.

Only after identification and recognition of a breed's health problems, further measures as modern DNA diagnostics and epidemiological techniques can be deployed to collectively and systematically work towards healthier dog breeds. So, in order to develop an effective policy, breeders should first have insight in the frequency of disease in their breed population, as well as the degree in which harmful breed specifications play a role in this. Such significant knowledge about the cumulative incidence of breed-related diseases and harmful breed specifications in Dutch breeds is still lacking up to this day.

### 1.3. Research

To fill this need, a Expertise Centre in this matter has been set up in 2013, on behalf of the Dutch Ministry of Economic Affairs, within the Faculty of Veterinary Medicine, Department of Clinical Sciences of Companion Animals of the Utrecht University. This Expertise Centre Genetics of Companion Animals, coordinated by Prof. Dr. J. Rothuizen, cooperates with i.a. the KNMvD (Royal Dutch Veterinary Society) and the kynological board of 'Raad van Beheer' (RvB). As its name entails, The Expertise Centre centers all scientific knowledge and research development on veterinary genetics, population genetics and molecular genetics at one place within the Utrecht University. From here, research will be communicated to the public via the website of the Faculty of Veterinary Medicine and the National Center of Information on Companion Animals (LICG) [17,18].

The first project of the Expertise Centre is to give insight in the status of breed-related health problems in the Netherlands, by providing an overview of diseases in all breed populations. Since pet owners firstly approach the local veterinarian when his/her pet is ill, patient data from primary care veterinary practices clearly reflect the health status of pet breeds through time. Through a pilot study in April 2014, in which textual consult descriptions from primary care veterinary practices of four pet breeds had been collected by hand and were translated into usable incidence data, proved disease registration records of veterinary practices to be of good use for future research[19]. However, since this manual data collection was very time-consuming, a system that automatically gathers and saves disease electronic registration records in a central database, is now top priority of the Center of Expertise.

The disease registration database will generate incidence data that will play a key role in identifying and quantifying breed-related diseases and harmful breed specifications within the Netherlands, as a prerequisite for the development of proper policy towards improved well-being and sustainable health of pet breeds.

Monitoring the health of pet populations through standardized cumulative incidence data has never been done before in the world. In this research, the progressive process is described of creating and thoroughly organizing the disease registrations database of dogs and cats in the Netherlands. Also, the epidemiological analysis is described showing the future possibilities of the database once it will be fully employed.

The aims of this research were:

- To develop a method for analysis of the newly set up national incidence database
- To combine the outcome with the qualitative methods of analysis
- To set up a template for reporting the combined information suitable for veterinary practice and animal breeders

# Materials and methods

## 2.1. Location

The research was done within the 'Expertise Centre Genetics of Companion Animals' located within the faculty Medicine of Companion Animals of Utrecht University, The Netherlands.

## 2.2. Qualitative analysis

To test the usability of incidence data to gain insight in the health status of a breed, the Expertise Centre started a qualitative research using four sample dog breeds. Firstly, a general insight in the health status of these breeds was gained by consulting all scientific literature on breed-related inherited diseases and harmful breed specifications, and by interviewing expert-specialists of the Utrecht University Clinic of Companion Animals (UKG). After this, the UKG patient database was analyzed to evaluate the presentation of these breeds in all specialized policlinics of the UKG .

Since UKG patients belong to a subpopulation that suffer more severe disease or disease that are harder to diagnose or treat, the UKG database does not reflect the actual incidence of disease within breed populations. However, by comparing the patient data from the UKG policlinics to the earlier findings from literature and the opinion of specialist-experts, this research does show if disease incidence is a legitimate variable to measure the health of breeds.

In this research, the same qualitative analysis was performed on the Cairn Terrier population in the Netherlands. Eventually, results have been described in an official report that can be sent to the Cairn Terrier's breed associations upon request. This will give them an insight in the health of their breed, and points out the areas of improvement.

### 2.2.1. Literature research

The sources used for literature research were scientific articles, veterinary books, reports from breed associations, websites of genetic laboratories for companion animals and, if available, databases on companion animal genetics of foreign veterinary clinics.

The found breed-related diseases were valued and categorized by their relevance to these populations in the Netherlands, as well as by source reliability. This resulted in an overview of A-, B- and C-listed diseases, A-listed diseases being very likely over-represented in the breed and important for the Dutch population of Cairn Terriers, B-listed diseases valued as (likely) inherited and present in foreign populations of the breed but not (yet) proven present in the Netherlands and therefore important for future monitoring, and C-listed diseases that, despite being mentioned the literature, is only mentioned in small number or case reports, seem not to be inherited and are therefore of no importance for future monitoring in the Netherlands.

### 2.2.2. Interviewing veterinary specialists

To investigate the considered prevalence of breed-related disease as found in the literature, and to confirm their relevance for the Dutch Cairn Terrier population, veterinary specialists of the Utrecht University Medicine of Companion Animals (UKG) were interviewed. For every specialized discipline, one organ system expert and European Board of Veterinary Specialization approved specialist was interviewed on the relevance of the A-, B- and C-listed diseases for all four breeds, using a standardized questionnaire (see attachment 5.1.). The organ specialists represented the organ systems also used in the UKG database analysis (see below). According to the results from these interviews, the list of disease derived from the literature research was adjusted and/or complemented.

### 2.2.3. UKG database analysis

Subsequently, the UKG patient database was analyzed. The frequency at which the investigated four dog breeds presented itself in the specialized UKG disciplines was investigated during a time-span of five years, or, if this led to too little data, this period was extended to ten years. Afterwards, these frequencies were compared to a control group existing of mixed-bred dogs. Since these animals are most varied regarding genotype (heterogeneity) and phenotype, comparing these to the four investigated breeds will clearly show any overrepresentation. Since the UKG does not differentiate between mixed-bred and cross-bred, cross-bred dogs were also incorporated to this control group.

The number of consults for the four dog breeds as well as for the control group were extracted from the following specialized UKG policlinics: general surgery, cardiology and pulmonology, dermatology, endocrinology, gastro-enterology, hematology, hepatology, internal medicine, otorhinolaryngology, nephrology, neurology, oncology, ophthalmology, orthopedics and neurosurgery, emergency, dental medicine, urology and reproduction. Animals that were brought into the UKG for research participation only, or visited for a breeding suitability medical check-up only, were excluded from the database.

### 2.2.4. Data analysis

Using the statistical software SPSS, the odd's ratio (OR) of all policlinics for the four investigated breeds was calculated. This OR means the chance that a dog from one of these breeds attended one of the UKG policlinics, compared to the chance of a control animal. When the OR equals 1, this chance is the same for both groups. However, when OR is bigger than 1, this risk is higher for the breed. Overrepresentation of a breed within an UKG policlinic was defined at OR>1,5, meaning that the purebred animal has a 50% higher risk of attending this policlinic then a mixed-breed dog. For this statistical analysis, a confidence interval of 95% was used.

In case a breed was significantly overrepresented within a particular UKG discipline, the diagnoses most frequently found within this policlinic were determined for this breed. If needed, these diseases were added to or removed from the A-, B- and C-list of most important diseases for every breed.

Also, to test for any age differences, the patient's mean, median, minimum and maximum age was determined at the moment of the first consultation. These age variables were compared to the ones of the control group

using a non-parametric test. Also the gender distribution was analyzed (including whether or not the dogs were intact).

## 2.3. QUANTITATIVE RESEARCH

To draw reliable conclusions regarding breed health that reflect the entire breed population, it is essential to combine the results of qualitative analysis with quantitative data. This is why the Expertise Centre focused on investigating the actual incidences of breed-related disease in the Netherlands. To do this, veterinary primary care disease registration records are essential and should therefore play a leading role [25].

### 2.3.1. PETscan

So, for this research, patient data from the practice management systems (PMS-system) of primary care veterinary practices were collected. Since this data collection had to be an automatically driven process, software was developed that could be integrated into these PMS-systems, consequently transferring the data of interest from the veterinary practices to a database managed by the Center of Expertise.

The software for this central disease registration system was developed in cooperation with an ICT firm and was named PETscan[20]. PETscan is an online computer program that pops up in the PMS-system of a veterinarian as a last step of a patient's registration. When PETscan is opened, the patient's personal data (species, breed, age, sex, chip-number) and the consultation date is automatically filled in as registered in the PMS-system. Subsequently, the veterinarian will need to define his/her patient's diagnosis through a couple of mouse clicks, which is only a matter of seconds. An important advantage of PETscan is that it not just transfers the entered veterinary data, it also serves as a helpful diagnostic tool for veterinarians. When using PETscan, the veterinarian is directed from the involved organ system until the in-depth diagnosis through a 'decision tree'-like system. This system functions in accordance with the veterinary method of clinical reasoning and decision-making. The format of PETscan is depicted in figure 1.

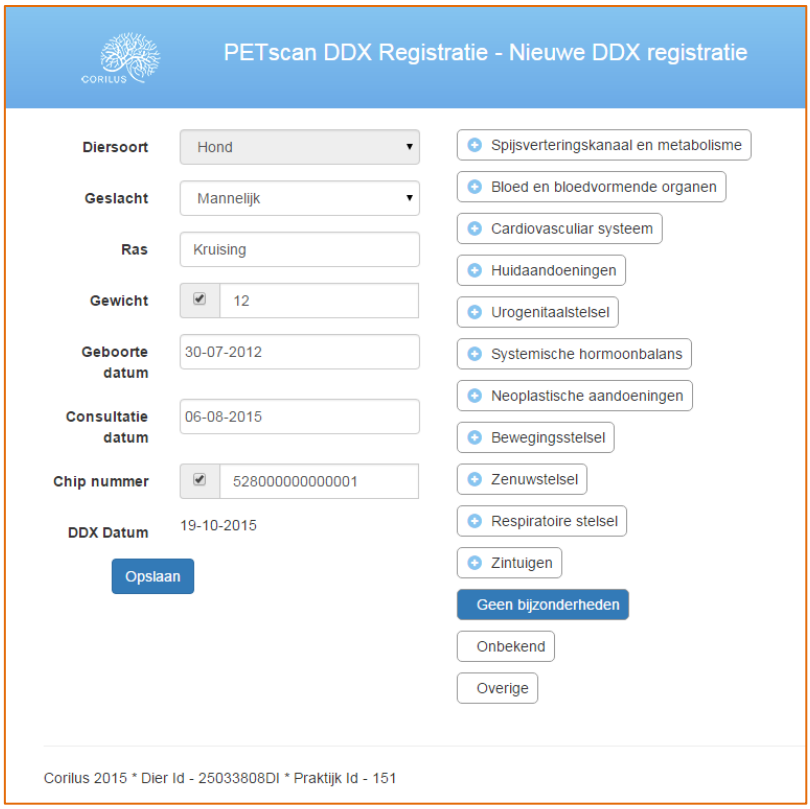


**Figure 1:** PETscan DDX registration program.

It is not always possible for primary care veterinarians to find a validated in-depth diagnosis for a patient. Subsequently, this would lead to an unfinished diagnosis in PETscan. However, the local veterinarian is very well able to indicate the anatomical location or the organ system involved in the disease, and in many cases also a further sub-specification of the disease. In these cases, the decision tree-like system of PETscan enables these important diagnostic findings to be registered as well. This diagnostic decision-tree is shown in figure 2.

Subsequently, PETscan automatically downloads these patient registration records from primary care veterinary practices to an up-to-date cumulative incidence database within the Expertise Centre Genetics of Companion Animals.

Two PMS-suppliers, Corilus and Vetsware, have already committed in the project of the Center of Expertise, by integrating PETscan as an optional tool in their PMS-system. If all associated veterinary practices would systematically take PETscan into service, this will cover approximately 40% of all primary care veterinary practices (n=275) in the Netherlands. Moreover, it is expected that in 2016 almost all Dutch primary care veterinary practices will participate in the project as collaborations with other PMS software firms are ongoing.

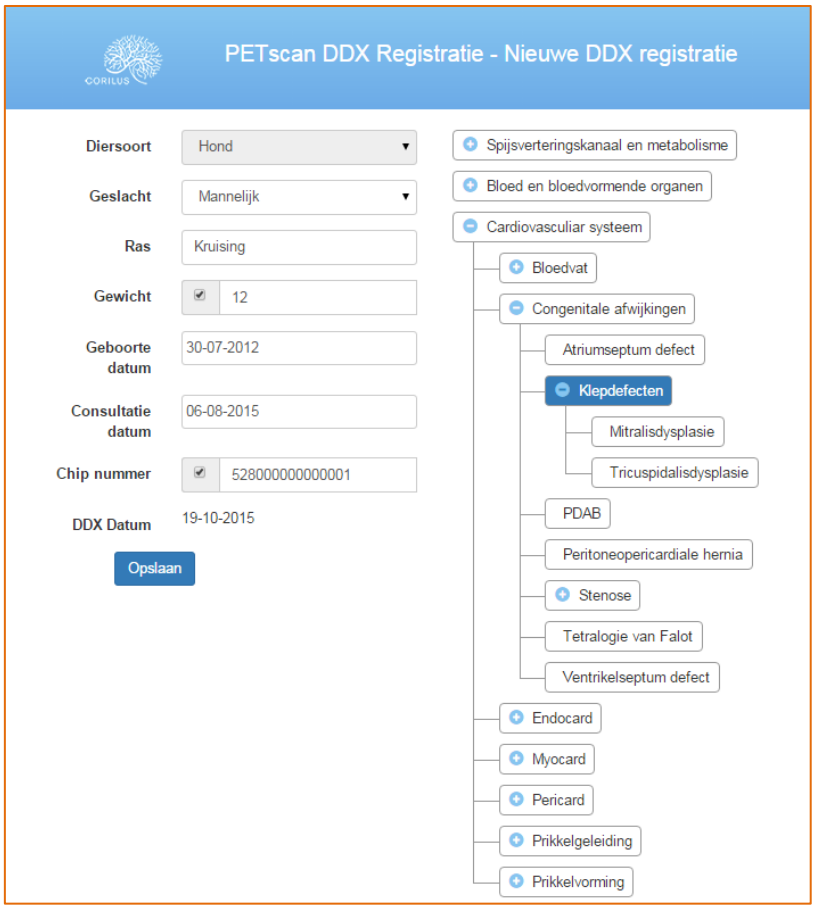


Figure 2: Depiction of the diagnostic decision-tree in PETscan.

### 2.3.2. Incidence database

As described earlier, PETscan downloads disease registration records from veterinary practices to the incidence database, initially displayed in Microsoft Excel 2010. This is a constant process, which means data will be added as fast as patients attend their veterinarian. This will soon generate a voluminous dataset that needs a proper organization no enable targeted analysis. So, data must be logically organized, which can be done according to either of the variables: diagnosis, breed, age, sex, reproduction status (intact or neutered) or weight.

When organizing the data according to diagnosis, it is important to consider the diagnostic feasibility level of a primary care veterinarian. This means that it should be possible to trace back validated in-depth diagnoses to their sub-specified diagnosis or even the underlying involved organ system or anatomic location. Therefore, the functionality of coding all organ systems and (sub)diseases in the PETscan disease list is investigated. Also, the grouping of closely related diseases into 'buckets' is valued, to see if this creates a more clear and faster overview of the breed-related diseases. For example, when a patient gets diagnosed with disease involving the locomotion system, this date is scored 'QM'. If it is known that this disease concerns the hip of the patient, the date is coded 'QMXX01'. And, if this patient has been diagnosed with hip dysplasia, the date is coded 'QMXX01HD'. In this way, all hip dysplasia patients can be traced back to the overall group of patients with disease in the hip, or even the locomotion system in general. This will help to detect overrepresentations of a breed in not only one particular diagnosis, but also in a particular anatomical body part or organ.

In some cases, diagnoses that are closely related have been clustered in one 'bucket'. For example, all disease involving the patient's teeth are clustered in the bucket labeled 'teeth'. This enables searching more broadly and practically within the large set of data. Moreover, (most) disease in the same bucket often trace back to the same inherited trait, and should therefore be counted as the same breed-related health problem. All in all, using buckets generates a fast overview of a breed's problematic areas.

Another possibility for the database to find new insights in breed-related disease, is to group related breeds such as all shepherd-like breeds, and compare this group to mixed-breed dogs.

### 2.3.3. Epidemiological analysis

On this moment, the Expertise Centre is waiting for the data transfer from veterinary practices using the Corilus or Vetsware PMS-system to the incidence database. Therefore, it is not yet possible to show the epidemiological analysis using real data. However, this research will describe how epidemiological analysis works and how it will be collectively implemented as soon as the incidence database is fully active.

The first option is, in resemblance to qualitative analysis, to search the data for overrepresentation of breeds within a disease or grouped disorder. The organization of the data according to diagnosis or bucket, and subsequently calculating the incidence of disease for every breed, permits a quantitative assessment of over-represented disorders in a breed population when compared to incidences of a control population (see figure 3). This control group consists of all cross-breed and mixed-breed dog patients in the database. This can be done using a statistical Chi-squared test with significance of $p<0,05$. The same test and significance can be used to compare the incidences in purebred animals to look-alikes of that same breed.

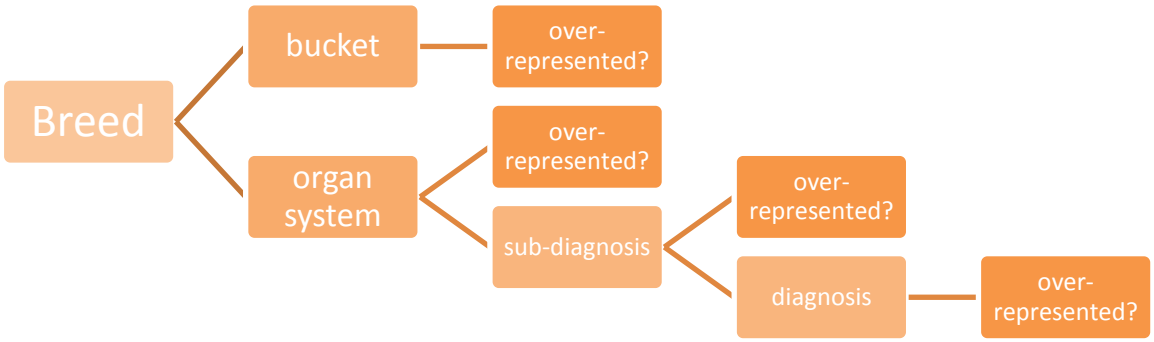


**Figure 3:** flow-chart to find overrepresentations in the database.

Another possibility when analyzing the database, is using a method called data mining. This scientific method is mostly known in non-medical databases consisting of raw numbers, but have been proven applicable to human medical databases as well, even using free-text data[21]. Data mining searches for statistical relations and/or patterns within all variables of a voluminous dataset. In this way, relevant information can be selected from large amounts of data. Data mining should be used according to the scientific guidelines, meaning that before every analysis, a proper falsifiable hypothesis should be set up, and only this hypothesis should be tested.

## 2.4. BREED REPORT

Although the quantitative analysis cannot yet be performed, the results from qualitative analysis already are of great importance to breeders in the Netherlands. It will offer them a clear and evidence-based overview of their breed's most important diseases and disorders, enabling them to develop a targeted breeding policy to eliminate these disease. Considering this, a template report was written describing the qualitative analysis, which can be adjusted for each breed. This breed-specific report can subsequently be sent to the belonging breeding association, and thereby distributed amongst all associated breeders. Also, an update on the progressions of the incidence project of the Expertise Centre was included in this report, informing on the future possibilities of the incidence database and its relevance to breeders.

# Results

## 3.1. Qualitative analysis

### 3.1.1. Literature study

In the literature a total of 45 diseases and disorders were described for the Cairn Terrier. These involved the following disciplines: liver, metabolism and hormones, bladder and urinary tract, eyes, gastrointestinal tract, jaws and teeth, blood and immune system, heart and vascular system, skin and coat, reproductive organs and mammary glands, kidneys, limbs and tail, spinal column, nervous system, and respiratory tract. Diabetes mellitus and congenital portosystemic shunting were listed as category A disease. The complete list of disease and disorders found in the Cairn Terrier breed can be found in attachment 5.2.

### 3.1.2. Veterinary experts

Four expert-specialists of the UKG have declared the Cairn Terrier as a common patient in their discipline, being gastro-enterology, dentistry, ophthalmology and hepatology. However, in the first two disciplines mentioned, the expert considered this being just a minor difference from other breeds. Since these problematic areas were not scientifically supported in the literature, it was chosen not to change the diseases list generated from the literature in response to these interviews.

### 3.1.3. UKG Database

Analysis of the UKG database during the period 2009-2013, the Cairn Terrier was overrepresented (OR > 1,5 and p < 0,05) in the UKG disciplines hepatology and ophthalmology (see table 1).

**Table 1:** Odds ratio per discipline for the Cairn terrier compared to mixed-breeds (2009-2013)

| Discipline | OR (CI 95%) | p-value (Fisher's exact) |
|---|---|---|
| Hepatology | 4.329 (2.521-7.434) | 0.000 |
| Opthalmology | 1.728 (1.130-2.641) | 0.015 |
| Gastro-enterology | 1.548 (0.542-4.419) | 0.343 |
| Endocrinology | 1.339 (0.569-3.3151) | 0.461 |
| Cardiology and pulmonology | 1.234 (0.632-2.408) | 0.577 |
| Emergency | 1.156 (0.776-1.722) | 0.461 |
| Neurology | 1.133 (0.581-2.207) | 0.720 |
| Reproduction | 1.089 (0.332-3.575) | 0.755 |
| Internal medicine | 0.883 (0.440-1.769) | 0.866 |
| Nephrology | 0.869 (0.114-6.619) | 1.000 |
| Oncology | 0.687 (0.297-1.587) | 0.464 |
| Orthopedics and neurosurgery | 0.557 (0.289-1.073) | 0.080 |
| Dental medicine | 0.541 (0.073-4.022) | 1.000 |
| Urology | 0.480 (0.065-3.554) | 0.717 |
| General surgery | 0.478 (0.256-0.894) | 0.019 |

| | | |
|---|---|---|
| Otorhinolaryngology | 0.434 (0.136-1.388) | 0.208 |
| Dermatology | 0.347 (0.109-1.104) | 0.060 |
| Haematology | 0.299 (0.041-2.185) | 0.367 |

Within the 19 Cairn Terrier patients with liver problems, a congenital portosystemic shunt was most frequently diagnosed. This diseases was diagnosed 7 times, of which 2 times as a probable diagnosis.

29 Cairn Terriers attended the UKG ophthalmology discipline, of which 10 patients were diagnosed with glaucoma. In 6 of these patients, this glaucoma was concerned secondary to an underlying disease or disorder.

The mean age of Cairn terrier hepatology patients was 0,2 years, compared to 5,1 years for control group patients. As seen in table 3, this age difference was not significant (p>0,05).

For ophthalmology Cairn terrier patients the mean age was 8,6 years, compared to 7,1 years for control group patients. As the p-value was smaller than 0,05, this age difference was significant (table 3).

Because the dataset for this analysis consisted of a relatively small number of Cairn terriers, the analysis was repeated for the spine-span of 10 years , from 2004 to 2013. In the resulting data, a total number of 403 cairn terriers had attended the UKG. Again, an overrepresentation (OR > 1,5 and p < 0,05) of the Cairn Terrier was found in the UKG disciplines hepatology and ophthalmology (table 2).

**Table 2:** Odds ratio per discipline for the Cairn terrier compared to mixed-breeds (2004-2013)

| Discipline | OR (CI 95%) | p-value (Fisher's exact) |
|---|---|---|
| Hepatology | 4.648 (3.316-6.514) | 0.000 |
| Opthalmology | 1.666 (1.273-2.181) | 0.000 |
| Gastro-enterology | 1.285 (0.661-2.500) | 0.453 |
| Endocrinology | 1.241 (0.762-2.020) | 0.418 |
| Nephrology | 1.220 (0.480-3.101) | 0.606 |
| Urology | 1.069 (0.488-2.345) | 0.836 |
| Cardiology and pulmonology | 0.975 (0.587-1.622) | 1.000 |
| Reproduction | 0.973 (0.419-2.263) | 1.000 |
| Emergency | 0.955 (0.727-1.255) | 0.784 |
| Haematology | 0.887 (0.383-2.056) | 1.000 |
| Internal medicine | 0.788 (0.509-1.220) | 0.308 |
| Dermatology | 0.769 (0.471-1.257) | 0.364 |
| Otorhinolaryngology | 0.723 (0.424-1.234) | 0.278 |
| Neurology | 0.649 (0.359-1.174) | 0.162 |
| Orthopedics and neurosurgery | 0.611 (0.405-0.920) | 0.018 |
| Dental medicine | 0.606 (0.220-1.672 | 0.406 |
| General surgery | 0.566 (0.390-0.821) | 0.002 |
| Oncology | 0.482 (0.236-0.988) | 0.041 |

The most frequently diagnosed liver disease for the 53 cairn terriers was the congenital portosystemic shunt. This was diagnosed in 18 patients, of which 6 times as a probable diagnosis.

74 Cairn Terriers attended the UKG ophthalmology discipline. 16 patients were diagnosed with glaucoma, in 6 cases secondary to an underlying disease. Another 10 patients were diagnosed with cataract.

The mean age of Cairn terrier hepatology patients was 0,9 years, compared to 4,3 years for control group patients. As seen in table 3, this age difference was not significant (p>0,05).

For ophthalmology Cairn terrier patients the mean age was 8,5 years, compared to 7,1 years for control group patients. This age difference was not significant (p>0,05), as seen in table 3.

**Table 3:** Mean, median, minimum and maximum age (in years) for the Cairn terrier and mixed-breeds for the Cairn terrier's disciplines of overrepresentation

| | Mean | Median | Minimum | Maximum | p – value |
|---|---|---|---|---|---|
| Hepatology Cairn terrier (2009-2013) | 3.6 | 0.2 | 0.1 | 13.2 | 0.116 |
| Hepatology mixed-breed (2009-2013) | 5.1 | 4.1 | 0.2 | 15.0 | |
| Ophthalmology Cairn terrier (2009-2013) | 8.5 | 8.6 | 1.8 | 14.2 | 0.049 |
| Ophthalmology mixed-breed (2009-2013) | 7.0 | 7.1 | 0.1 | 19.2 | |
| Hepatology Cairn terrier (2004-2013) | 4.2 | 0.9 | 0.1 | 13.4 | 0.114 |
| Hepatology mixed-breed (2004-2013) | 5.5 | 4.3 | 0.2 | 15.2 | |
| Ophthalmology Cairn terrier (2004-2013) | 8.5 | 8.7 | 1.6 | 15.3 | 0.077 |
| Ophthalmology mixed-breed (2004-2013) | 7.1 | 7.3 | 0.1 | 17.3 | |

The following gender distributions were found for the Cairn terrier in hepatology:

- 2009-2013: 10 intact males, 2 neutered males, 6 intact females, and 1 spayed female.
- 2004-2013: 17 intact males, 7 neutered males, 18 intact females, and 11 spayed females.

The following gender distributions were found for the Cairn terrier in ophthalmology:

- 2009-2013: 6 intact males, 8 neutered males, 5 intact females, and 10 spayed females.
- 2004-2013: 21 intact males, 15 neutered males, 11 intact females, and 27 spayed females.

### 3.1.4. Overview

The results from the UKG database analysis were combined with the results the literature study, to create a final overview of the most important disease and disorders for the Dutch population of Cairn terriers. These disease were categorized according to the attended UKG discipline or involved organ system (see table 4). In category A, congenital portosystemic shunting and diabetes mellitus were listed. Despite the fact that no overrepresentation was found for the Cairn terrier in the UKG discipline of endocrinology, diabetes mellitus was not removed from category A. This was because the disorder was considered to be both well-diagnosable and well-treatable by primary care veterinarians, and will therefore not attend the UKG often. Since cataracts and glaucoma were frequently found in Cairn terriers in the UKG, these disease were both scaled up one category, cataract now being a B-listed disease and glaucoma entering the A-list of most important diseases for the Cairn Terrier in the

Netherlands (table 4). The complete list of A-, B- and C-listed disease for the Dutch population of Cairn terriers can be found in attachment 5.2.

Table 4: Important disease and disorders for the Dutch population of Cairn terriers

| Organ system | Discipline | Disorder |
|---|---|---|
| Liver | Hepatology | Congenital portosystemic shunting |
| Metabolism and hormones | Endocrinology | Diabetes Mellitus |
| Eyes | Ophthalmology | Glaucoma |

## 3.2. QUANTITATIVE RESEARCH

### 3.2.1. PETscan

The list of organ system categories and belonging (sub)diseases that is used in PETscan can be found in attachment 5.3.

### 3.2.2. Incidence database

Within the database, all organ systems up to all diseases that can be found in this organ system were chronologically encoded. Also, parent codes were assigned to make sure diagnoses could be linked back to their sub-diagnoses, and even further back to the involved organ system. This codification method is depicted in table 5. The full list can be found in attachment 5.3.

Closely related diseases were grouped in the same bucket. This was done considering the feasibility of a primary care veterinarian to diagnose a (group of) disease and in which extent diseases can be traced back to the same inherited trait. Also genetically related breeds (sometimes referred to as 'dog types') were combined into buckets. This enables fast linkage of overrepresented disease in one breed, to the importance of this disease to other breeds from the same dog type. An example of the disease list grouped into buckets is depicted in table 5. The entire list can be found in attachment 5.3. It can be seen that using buckets generates a fast overview of a breed's problematic areas.

Table 5: chronological codification of all diagnoses, subsequently grouped into buckets.

| **Diagnose** | **Code** | **Parent code** | **Bucket code** | **Bucket name** |
|---|---|---|---|---|
| **Bewegingsstelsel** | **QM** | | QM | bewegingsstelsel |
| **Heup** | **QMXX01** | QM | QMXX01 | Heup |
| o Heupdysplasie | QMXX01HD | QMXX01 | QMXX01 | Heup |

| | | | | |
|---|---|---|---|---|
| o Avasculaire femurkopnecrose (Calvé-Legg-Perthes) | QMXX01AF | QMXX01 | QMXX01 | Heup |
| o Osteoarthrose/-arthritis | QMXX01OA | QMXX01 | QMXX01 | Heup |
| o Heupluxatie | QMXX01HP | QMXX01 | QMXX01 | Heup |
| o Fractuur | QMXX01FR | QMXX01 | QMXX01 | Heup |
| o Neoplasie | QMXX01NP | QMXX01 | QMXX01 | Heup |
| **Elleboog** | **QMXX02** | QM | QMXX02 | Elleboog |
| o Elleboogdysplasie | QMXX02ED | QMXX02 | QMXX02 | Elleboog |
| · Los processus anconeus (LPA) | QMXX02EDLA | QMXX02ED | QMXX02 | Elleboog |
| · Los processus coronoideus (LPC) | QMXX02EDLC | QMXX02ED | QMXX02 | Elleboog |
| · Osteochondrose dissecans (OCD) | QMXX02EDOD | QMXX02ED | QMXX02 | Elleboog |
| · Elleboog incongruentie (EI) | QMXX02EDEI | QMXX02ED | QMXX02 | Elleboog |
| · Medial compartment syndroom (MCD) | QMXX02EDMD | QMXX02ED | QMXX02 | Elleboog |
| o Osteoarthrose/-arthtitis | QMXX02OA | QMXX02 | QMXX02 | Elleboog |
| o Ellebooglуxatie | QMXX02EL | QMXX02 | QMXX02 | Elleboog |
| o Fractuur | QMXX02FR | QMXX02 | QMXX02 | Elleboog |
| o Neoplasie | QMXX02NE | QMXX02 | QMXX02 | Elleboog |
| **Knie** | **QMXX03** | QM | QMXX03 | Knie |

Also, disease that are not significantly overrepresented but do not lose interest because of e.g. high severity, might do show overrepresentation in comparison to mixed-bred dogs when combining all related dog breeds into one population.

## 3.3. Breed report

A report describing the health status of the Cairn Terrier according to this qualitative analysis was written on behalf of the Center of Expertise. On request, this report can be sent to the breed association to inform them about the health status of their breed, and as an update on the progressions in the incidence project of the Center of Expertise, informing breed associations on the future possibilities of the database. The full report can be found in attachment 5.4.

# Discussion

## 4.1. CONCLUSIONS

In this research it is concluded that the combination of qualitative and quantitative analysis of breed populations can generate reliable conclusions on the incidence of breed-related disorders and disease.

Qualitative analysis gives evidence-based insight in the most important health problems of a breed, which can be further categorized based on importance to the Dutch breed population. Also, qualitative research shows which data and techniques are necessary for disease incidence measurement in quantitative analysis. The list of most important diseases and disorders within a breed that is generated from this analysis, is very useful for breeders in the Netherlands.

Subsequently, the incidence database of the Expertise Centre was created, and the possible organization methods were described. To create the database, PETscan was computed using a set list of all possible diseases. Data consisting of the variables species (dog or cat), breed, age, sex, reproduction status (neutered or intact) identification number and diagnosis, were automatically downloaded to the incidence database of the Center of Expertise.

To generate a clear overview, the data were organized using buckets. Closely related disease were grouped into buckets of more or less specified health problems of varied size. This enables fast detection of breed over-representations. Also, this avoids the exclusion of sub-diagnoses made by first-line veterinarians.

Overrepresentation of a breed within a disease or group of diseases (bucket) should be analyzed as soon as the database is fully active. This can be done by comparing the incidence of a specific diagnosis, sub-diagnosis or involved organs system in breeds to this incidence in mixed-bred dogs. Data mining was also considered a method of analyzing the database. This scientific method searches for statistical relations and/or patterns within a voluminous dataset. This method is time-consuming and complex, but prevents exclusion of data and should therefore generate reliable results.

To summarize, this research proves the incidence database to be of great importance to the Dutch breeding sector and veterinary medicine. This epidemiological project of the Expertise Centre Genetics of Companion Animals generates usable breed-related disease incidences, making way for further DNA- and epidemiological development that will eventually lead to a collaborative formation of evidence-based breed-specific breeding policies that will lead to healthier breeds in the Netherlands.

## 4.2. EVALUATION OF THE QUALITATIVE RESEARCH

The qualitative research provides information on the relative importance of particular disorders to breeds in the Netherlands. However, no conclusions can be made regarding the prevalence of these disorders within the Dutch population of the breed.

Also, the UKG patients used in this research do not reflect the overall breed population. It only concerns animals that suffer disease that are more difficult to diagnose and/or to treat, and are therefore redirected by the first-line veterinarian. Important hereditary disorders will be missed in the analysis of this database, as a primary care veterinarian is very well able to diagnose and treat most diseases.

Lastly, it should be taken into account that the number of patients of certain breeds that visited the UKG was often fairly low. This could relate to the breed being relatively healthy, but is more likely to be explained by the total population of the breed being smaller. Investigating a small amount of patients, makes results of analysis less reliable, as only a few patients less or more can make a great difference in significant overrepresentation of a breed within a UKG discipline.

When discussing the results of the qualitative analysis of the Cairn terrier breed specifically, some aspects should be taken into account when drawing conclusions. According to the literature study, diabetes mellitus was an important disease for the Cairn terrier. However, analysis of the UKG database did not show an overrepresentation of the breed in endocrinology, meaning that diabetes mellitus was interpreted as unimportant in this part of the research. Despite the fact that no overrepresentation was found for the Cairn terrier in the UKG discipline of endocrinology, diabetes mellitus was not removed from category A. This was because the disorder was considered to be both well-diagnosable and well-treatable by primary care veterinarians, and will therefore not attend the UKG often.

Cataract was found to be an important disease in the analysis of the UKG database, but only in a ten year period. It was therefore moved from category C to category B instead of A, as ten years ago is less recent, and therefore, less relevant to the population of Cairn terriers in the Netherlands on this moment.

## 4.3. EVALUATION OF THE QUANTITATIVE RESEARCH

In this research, the progressive process is described of creating yet thoroughly organizing a disease registration database of dogs and cats in the Netherlands. Also, the epidemiological analysis is described that will be performed as soon as data enter the database.

The research revealed that disease registrations from primary care veterinary clinics generate usable insights in the health status of pet breeds. The access to cumulative incidence data of disease in breeds, provided by the Center of Expertise, is of great importance for the breeding sector in the Netherlands. It enables targeted research on each specific breed's most important disease and disorders. When all epidemiological and genetic knowledge is centered in one place of expertise, this enables breed-targeted further research on how to eliminate these diseases from the populations. As disease registrations are automatically gathered and constantly monitored, new breed-specific and evidence-based breeding policies can immediately be followed and evaluated through time. The Expertise Centre Genetics of Companion Animals will play the main supervisory and communicative role in this project, instructing and guiding all partners in the Dutch breeding sector in the process towards healthier and more sustainable breeds.

### 4.3.1. PETscan

When evaluating the disease list used in PETscan, it was mentioned that neoplasia will need to be valued differently than all other diseases. The diagnosis neoplasia was logically categorized under every belonging organ system, since it can occur in every location within the body. However, when a veterinarian suspects his/her patient of a neoplasia, most anatomical locations will enable the relatively simple procedure of performing an DNAB that can be sent to a diagnostic laboratory. This results in a relatively fast in-depth diagnosis of the neoplasia, which is not represented in the disease list of PETscan. Therefore, it was concluded that the diagnoses 'neoplasia' should be more specified to generate more specific incidences of these diseases. Cooperation with the National Cancer Fund for Animals (NKFD) is expected to generate a list of all specified neoplasia known in dogs to be incorporated in the disease list of PETscan in the near future.

### 4.3.2. Incidence database

To facilitate the option of generating a fast overview of a breed's problematic areas, related disease as well as related breeds in the database are clustered into groups, or 'buckets'. However, this clustering and grouping of data also has its downsides. For instance, it may lead to data generalization, causing extinction of effects that could have been detected otherwise. Concerning the clustering of breeds, it should be noted that there is a risk for significant breed-related problems to become concealed when searching for significance in the breed group as a whole. Therefore, it is important that groups and buckets must be chosen carefully, and that analysis should be performed on both grouped data as well as the dataset in general.

### 4.3.3. Epidemiological analysis

Lastly, epidemiological analysis of the finalized database should indicate the specific health problems of breed populations in the Netherlands.

The promising analysis of voluminous datasets as the incidence database by the method of data mining, should however be used carefully. A disadvantage of data mining is the possibility of bias due to the sophism 'cum hoc ergo propter hoc'. This means that a significant correlation is found between two variables that are actually not at all related. This risk should be avoided by the use of strict hypotheses before testing for any relations within the dataset.

### 4.3.4. Uniqueness

As said before, the incidence database of the Expertise Centre is a pioneer in its field. Attempts for practice-based disease surveillance databases have been made by several other veterinary institutions in the world, for example in the UK[22], but these are our outweighed by the extent of the project of the Center of Expertise.

Firstly, the use of PETscan software for first-line veterinary practices enables and fully automatic collection of patient registration records throughout the country. Moreover, the program offers a complete and standardized list of all diseases known in dogs, so every patient can be properly registered by the veterinarian.

In case a first-line veterinarian cannot find an in-depth diagnosis for its patient because it suffers a more severe or diagnostically complex disease, the patient often gets redirected to the more specialized and well-equipped University Clinic of Companion Animal Health (UKG) of the Utrecht University. To incorporate diagnoses made in the UKG into the incidence database, the Expertise Centre will link data output from primary care veterinary practices to the UKG patient database. The same goes for diagnoses made in pathological institutes throughout the country, which will also be incorporated in the incidence database. In this way, in-depth diagnoses from the UKG database or pathological institutes can complete patient data from first-line veterinary practices, without the particular patient being counted twice and therefore biasing the disease incidences.

Another aspect that gives the incidence database its uniqueness, is that it will distinguish between pedigree bred animals and so called 'look-alikes'. Look-alikes are animals that were bred pursuing the same breed standard as used by pedigree breeders, but are bred by amateurs that are not associated to official breeding clubs. This means that these breeders are not subjected to the breed association's strict breeding regulations and guidelines. As such, look-alike breeders have no obligation for central documentation of pedigrees, which may lead to breeding with animals with a similar descent. This explains that look-alikes are expected to generate different disease incidences than pedigree animals. Considering that the vast majority of some popular dog breeds in the Netherlands actually consists of these look-alikes, the matter has been a growing concern for pedigree breeders, veterinarians and breeding authorities as Raad van Beheer, which urges the need for scientific evidence of its consequences[23]. RvB has recently started a Fairbreed project ('Fairfok') in cooperation with Utrecht University, that will investigate the problem of look-alikes and will help working towards healthier breeds in the Netherlands by setting up breeding measures and advice[23]. Incorporating the distinction between pedigree and look-alike animals in the incidence database of the Center of Expertise, will be an important contribution to this Fairfok project.

## 4.4. FURTHER RESEARCH

Without the use of DNA-diagnostics, effectively reducing breed-related disease is an impossible mission. Future research should therefore focus on relating clinical incidences of breed-related disease to DNA of breed animals. In 2013 the Dutch DNA-database for dog breeds was put in service. This project was part of the research program Companion Animal Welfare, that was financed by the ministry of Economic Affairs. The project is administered within Raad van Beheer and allows access to all breeding associations. By analyzing the DNA of breed animals in relation to the incidence database, specific DNA mutations can be traced that cause expression of these breed-related inherited diseases and harmful breed specifications. Subsequently, DNA-tests can be developed to screen breed populations for presence of these DNA mutations.

The Expertise Centre is the central location of epidemiologic and genetic expertise. It will play the main communicative and supervisory role by providing DNA-tests and research results that can be used in new breeding policies. These breeding policies should be optimized for each individual breed, in consideration with the character and complexity of the genetic mutations causing breed-related disease, as well as with the genetic heterogeneity of the breed population. The Expertise Centre will supervise the implementation of these policies. The veterinarian will play a vital role in the guidance of breeders in these policies.

Also, the broad applicability of the incidence database generally serves the veterinary sector as a whole. The quantitative incidence data on pet breeds can be used to create new policy guidelines and serves as a quality incentive to veterinary medicine in the Netherlands. Also, as patient data are registered over time, overall performance of veterinary practices, or even individual veterinarians, can be analyzed at different levels of specification. Moreover, future expansion of PETscan may create new possibilities for veterinary practice-based research. For example, incorporating registration of medicine use by primary care veterinarians. This will not only generate usable data on medicine use in veterinary practices in the Netherlands, it will also serve as a valuable tool to evaluate for example side-effects of particular medicine. This feedback can be used in the overall quality policy of good veterinary practice. Moreover, research on this matter will also be of interest to the veterinary industry and even the government.

Another advantage of this DNA-technology, is its potential relevance to translational medicine. This approach focuses on the close resemblance of many veterinary and humane diseases. Molecular genetics in breed populations can gain new insights for complex humane disease of which genetic backgrounds were so far undiscovered. Therefore, a trend is seen in the field of humane biomedical research of increasing strategic interest in veterinary genetics.

These factors once again stress the significance of this research to science, the breeding sector, veterinary medicine and the society. Implementation of the incidence database will identify a breed's health problems, enabling further genetic and epidemiological research that will eventually develop new, evidence-based breeding policies, monitoring the effects of these policies, and help to detect epidemiological trends in all breed populations. With these policies, breeders, breeding authorities veterinarians and all other parties in the sector will be able to work collectively and systematically towards healthier dog breeds in the Netherlands.

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# Attachments

### Attachment 5.1.

The questionnaire used for veterinary expert opinions in qualitative analysis.

### Attachment 5.2.

List of important disease and disorders in the Dutch population of Cairn terriers

### Attachment 5.3.

List of organ system categories and belonging (sub)diseases as used in PETscan, and their codifications and buckets.

### Attachment 5.4.

Report of the most common inherited disease and harmful breed characteristics of the Dutch population of the Cairn terrier.